Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 143 (2007) 182–188
veterinary parasitology
www.elsevier.com/locate/vetpar

Short communication

Biologic and genetic comparison of *Toxoplasma gondii* isolates in free-range chickens from the northern Pará state and the southern state Rio Grande do Sul, Brazil revealed highly diverse and distinct parasite populations

J.P. Dubey [a,*], N. Sundar [a], S.M. Gennari [b], A.H.H. Minervino [c], N.A. da R. Farias [d], J.L. Ruas [e], T.R.B. dos Santos [e], G.T. Cavalcante [a], O.C. H. Kwok [a], C. Su [f]

[a] *United States Department of Agriculture, Agricultural Research Service, Animal and Natural Resources Institute, Animal Parasitic Diseases Laboratory, Building 1001, Beltsville, MD 20705-2350, USA*
[b] *Faculdade de Medicina Veterinária e Zootecnia, Departamento de Medicina Veterinária Preventiva e Saúde Animal, USP, São Paulo, SP, Brazil*
[c] *Faculdade de Medicina Veterinária e Zootecnia, Departamento de Clínica Médica, USP, São Paulo, SP, Brazil*
[d] *Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, RS, Brazil*
[e] *Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, RS, Brazil*
[f] *Department of Microbiology, The University of Tennessee, Knoxville, TN 37996-0845, USA*

Received 9 June 2006; received in revised form 5 July 2006; accepted 1 August 2006

Abstract

The prevalence of *Toxoplasma gondii* in 84 free-range chickens (34 from the northern Pará state, and 50 from Rio Grande do Sul, the southern state) from Brazil, South America was determined. Antibodies to *T. gondii* were assayed by the modified agglutination test (MAT), and found in 39 (46.4%) of 84 chickens with titers of 1:10 in one, 1:20 in two, 1:40 in four, 1:80 in seven, 1:160 in five, 1:320 in six, 1:640 in eight and ≥1:1280 in six. Hearts and brains of 45 chickens with titers of 1:20 or less were pooled and fed to two *T. gondii*-free cats. Hearts and brains of 39 chickens with titers of 1:10 or higher were bioassayed in mice. Feces of cats were examined for oocysts. One cat fed tissues from 31 chickens with titers of less than 1:10 from Rio Grande do Sul shed *T. gondii* oocysts. *T. gondii* was isolated by bioassay in mice from 33 chickens with MAT titers of 1:20 or higher. All infected mice from 10 isolates died of toxoplasmosis. All 34 isolates (15 from Pará, 19 from Rio Grande do Sul) were genotyped using 11 genetic markers including SAG1, SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, a new SAG2 and Apico. Eleven genotypes were revealed for Pará isolates and seven genotypes for Rio Grande do Sul. No genotype was shared between the two geographical locations. These data suggest that *T. gondii* isolates are highly diverse and genetically distinct between the two different regions in Brazil that are 3500 km apart.

Keywords: *Toxoplasma gondii*; Chickens; *Gallus domesticus*; Free-range; Pará state; Rio Grande do Sul state; Brazil; Genotype

1. Introduction

The prevalence of *Toxoplasma gondii* infection in humans in Brazil is unusually high, reaching to 100% in some areas (Dubey and Beattie, 1988; Bahia-Oliveira et al., 2003; de Moura et al., 2006; Sobral et al., 2005).

* Corresponding author. Tel.: +1 301 504 8128; fax: +1 301 504 9222.
E-mail address: jdubey@anri.barc.usda.gov (J.P. Dubey).

0304-4017/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2006.08.024

In Erechim, which is located in the northern part of the southern state of Rio Grande do Sul, Brazil 17% of 1000 unselected humans examined were found to have ocular toxoplasmosis; this is the highest prevalence of ocular disease in any city of the world (Glasner et al., 1992). In a well controlled epidemiologic study of 131 persons with recently acquired ocular toxoplasmosis with demonstrable IgM antibodies in Erechim, and 110 matched uninfected controls indicated that working in the garden and eating lamb were two important risk factors for acquired toxoplasmosis (Jones et al., 2006). *T. gondii* DNA isolated from blood or ocular fluids of some of these patients indicated that unusual *T. gondii* isolates may have caused the ocular disease (Khan et al., 2006).

We have recently found that the isolates of *T. gondii* from Brazil are biologically and genetically different than those from North America and Europe (Dubey et al., 2002, 2003a,b, 2006a; Lehmann et al., 2004, 2006). In the present paper, we attempted to isolate, genotype, and compare *T. gondii* from chickens from the very north, Pará state, with chickens from Rio Grande do Sul, the very southern state of Brazil, approximately 3500 km apart one from other. For this study, we chose free-range chickens as the indicator for soil contamination with *T. gondii* oocysts because chickens feed from the ground. Direct detection of oocysts in soil is technically difficult and only 1% of cats are found shedding *T. gondii* oocysts at any time (Dubey, 2004).

2. Materials and methods

2.1. Naturally infected chickens

During February and March, 2006 a total of 84 free-range chickens were obtained for the present study. The Pará state chickens ($n = 34$) were from six municipalities (Castanhal—1°17′ 49.2″S and 47°5′ 19.2″W, Inhangapi—1°25′ 48.″S and 47°5′ 1.2″W, Marituba—1°2′ 18.0″S and 48°20′ 31.2″W, Santa Isabel do Pará—1°17′ 56.4″S and 48°09′ 39.6″W, Santarém—2°26′ 34.8″S and 54°42′ 28.8″W and Terra Alta—1°2′ 16.8″S and 47°54′ 28.8″W Table 1; Fig. 1).

The Rio Grande do Sul chickens ($n = 50$) were from 10 farms from five municipalities (Pelotas—31°46′ 19.2″S and 52°20′ 34.8″W, Capão do Leão—31°45′ 46.8″S and 52°29′ 02.4″W, Turuçu—31°25′ 19.2″S and 52°10′ 40.8″W, Canguçu—31°23′ 42.0″S and 52°40.0′ 33.6″W and Rio Grande—32°02′ 06.0″S and 52°05′ 56.4″W) with two farms from each municipality (Table 2; Fig. 1—only four municipalities are shown).

Chickens were purchased, killed by cervical dislocation, and samples of brain, whole heart, and blood were collected from each chicken, kept at 4 °C until sent

Table 1
Isolation of *T. gondii* from free-range chickens from Pará State, Brazil

Chickens			Isolation in mice			Genotype		
Expt no. and chicken no.	Farmhold location	MAT titer	No. infected[a]	No. died	Day of death	Isolate ID	SAG1, SAG2, SAG3, BTUB, GRA6[b]	c22-8, c29-2, L358, PK1, SAG2 (new), Apico
Tx 233								
5	Santarém	≥1280	4	3	14, 18, 19	TgCkBr107	I(4), III(4), III(4), III(4), II(4)	u-1, I, I, I, III, III
6	Santarém	1280	4	4	14, 16, 21, 23	TgCkBr108	I(4), III(4), III(4), III(4), II(4)	u-1, I, I, I, III, III
8	Santarém	320	4	4	14, 16, 16, 17	TgCkBr109	I(4), I(4), I(4), III(4), III(4)	II, III, I, III, II, III
9	Santaéem	320	4	1	14	TgCkBr110	I(4), III(4), I(4), III(4), III(4)	III, III, III, I, III, I
12	Santarém	640	4	3	21, 23, 23	TgCkBr111	I(4), III(4), III(4), III(4), III(4)	III, III, III, III, III, I
15	Santarém	≥1280	4	2	23, 23	TgCkBr112	I(4), III(4), III(4), III(4), III(4)	III, III, III, III, III, I
Tx 239								
4	Inhangapi	640	4	3	13, 15, 33	TgCkBr113	I(3), III(3), I(3), III(3), III(3)	III, III, III, I, III, III
5	Terra Alta	160	1	0		TgCkBr114	I(1), I(1), III(1), I(1), III(1)	II, I, III, III, II, I
7	Terra Alta	640	4	1	20	TgCkBr115	I(4), I(4), I(4), I(4), I(4)	II, I, III, I, I, III
9	Castanhal	20	1	0		TgCkBr116	u-1(1), II(1), III(1), III(1), II(1)	II, nd, II, II, II, I
12	Castanhal	640	4	4	19, 19, 19, 19	TgCkBr141	I(3), I(3), I(3), I(3), I(3)	u-1, I, I, III, I, III
14	Santa Isabel	80	4	1	24	TgCkBr142	I(4), I(4), I(4), I(4), I(4)	II, I, III, I, I, III
15	Santa Isabel	320	4	3	21, 23, 28	TgCkBr143	I(4), I(4), III(4), III(4), II(4)	u-1, III, III, III, II, I
16	Santa Isabel	80	1	1	21	TgCkBr144	I(1), I(1), III(1), I(1), II(1)	u-1, I, I, I, I, I
17	Marituba	160	4	1	20	TgCkBr145	I(4), I(4), I(4), I(4), I(4)	II, I, III, I, I, III

nd, data not available.
[a] Four mice were inoculated with tissues of each chicken.
[b] Numbers in parenthesis are the number of mice used separately for genotyping.

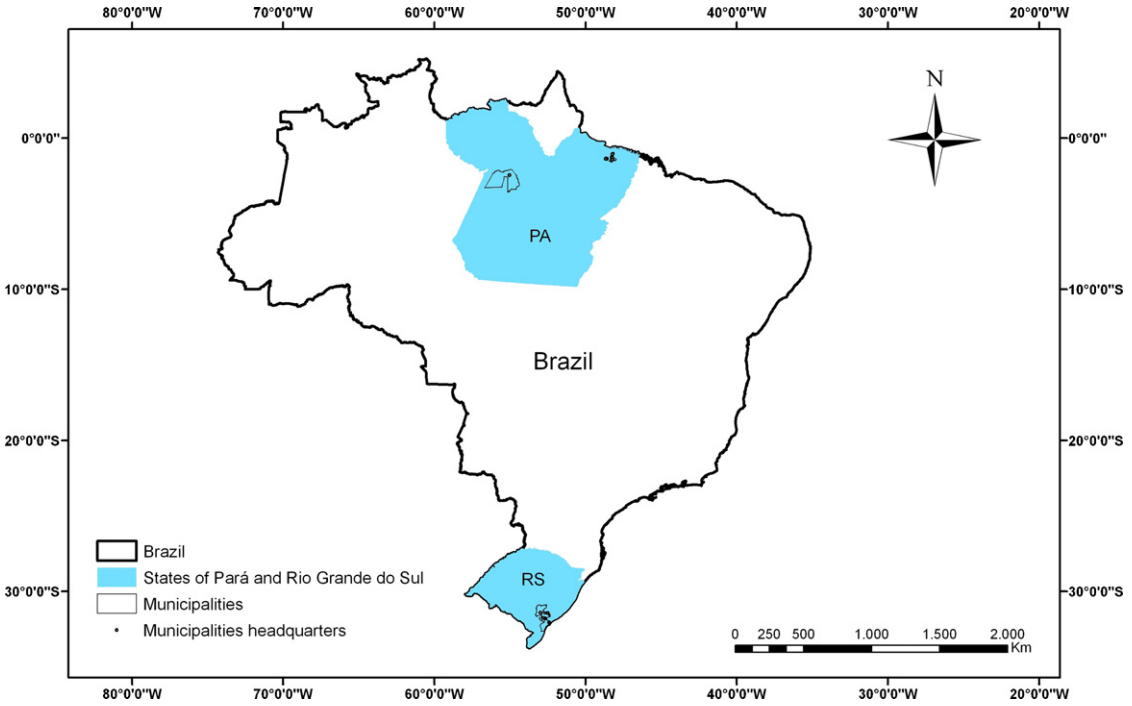

Fig. 1. Map of Brazil showing the sampled states, Pará (PA) and Rio Grande do Sul (RS).

with cold packs by air to Beltsville, MD. Five days elapsed between killing of chickens and receipt of samples at Beltsville. Samples were received in excellent condition.

2.2. Serological examination

Sera of chickens were tested for *T. gondii* antibodies using eight dilutions, from 1:10 to 1:1280 with the modified agglutination test (MAT) as described by Dubey and Desmonts (1987).

2.3. Bioassay of chickens for T. gondii infection

Tissues of all chickens were bioassayed for *T. gondii* infection. Brains and hearts of 39 (20 from Pará, 19 from Rio Grande do Sul) chickens with titers of 1:20 or higher were bioassayed individually in outbred female Swiss Webster mice obtained from Taconic Farms, Germantown, New York, as described (Dubey et al., 2002). Tissues were homogenized, digested in acidic pepsin, washed and homogenate inoculated subcutaneously into four mice (Dubey, 1998).

Brains and hearts from 45 (14 from Pará, 31 from Rio Grande do Sul) seronegative (MAT $<$ 1:10) chickens were pooled and fed separately to two *T. gondii*-free cats. Feces of the cats were examined for shedding of *T. gondii* oocysts 3–14 days post-ingesting chicken tissues as previously described (Dubey et al., 2002). Fecal floats were incubated in 2% sulfuric acid for 1 week at room temperature on a shaker to allow sporulation of oocysts and were bioassayed orally in mice (Dubey and Beattie, 1988). Tissue imprints of lungs and brains of mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled on day 41 post-inoculation (p.i.) and a 1:25 dilution of serum from each mouse was tested for *T. gondii* antibodies with the MAT. Mice were killed 43 days p.i. and brains of all mice were examined for tissue cysts as described (Dubey and Beattie, 1988). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues.

2.4. Genetic characterization for T. gondii

T. gondii DNA was extracted from the tissues of all infected mice from each group and strain typing was performed using genetic markers SAG1, SAG2, SAG3, BTUB and GRA6 as previously described (Dubey et al., 2006b). These five markers allow us to quickly characterize all samples and to identify potential mixed infection in chickens. One representative DNA extract from mice infected with the same chicken sample was

Table 2
Isolation of *T. gondii* from free-range chickens from Rio Grande do Sul, Brazil

Chickens			Isolation in mice[e]			Genotype		
No. TX 242	Farmhold location[a]	MAT titer	No. infected	No. died	Day of death	Isolate ID	SAG1, SAG2, SAG3, BTUB, GRA6	c22-8, c29-2, L358, PK1, SAG2 (new), Apico
1A1	Pelotas farm A	160	4	4	14, 14, 15, 15	TgCkBr146	I(4), I(4), I(4), I(4), I(4)	I, I, I, I, I, I
1A2	Pelotas farm A	320	4	4	14, 14, 15, 16	TgCkBr147	u-1(4), I(4), III(4), III(4), III(4)	u-1, I, I, III, II, I
1A3	Pelotas farm A	≥1280	4	4	14, 14, 15, 15	TgCkBr148	u-1(4), I(4), III(4), III(4), III(4)	u-1, I, I, III, II, I
1B1	Pelotas farm B	160	4	0		TgCkBr149	II or III(4), III(4), III(4), III(3), III(4)	I, III, III, I, III, III
1B2	Pelotas farm B	160	4	0		TgCkBr150	II or III(4), III(4), III(4), III(4), III(3)	I, III, III, I, III, III
1B3	Pelotas farm B	80	4	4	13, 15, 15, 15	TgCkBr151	u-1(4), I(4), III(4), III(4), III(4)	u-1, I, I, III, II, I
1B4	Pelotas farm B	20	4	1	20	TgCkBr152	II or III(4), III(4), III(4), III(4), III(4)	I, III, III, I, III, III
1B5	Pelotas farm B	320	2[b]	2	14, 16	TgCkBr153	I(2), III(2), III(2), III(2), III(2)	III, I, III, III, III, III
2B5	Capão do Leão farm B	80	4	4	14, 14, 15, 15	TgCkBr154	u-1(4), I(4), III(4), III(4), III(4)	u-1, I, I, III, II, I
4A1	Canguçu farm A	640	2[c]	0		TgCkBr155	u-1(2), III(2), III(2), III(2), III(2)	u-1, I, I, III, III, I
4A2	Canguçu farm A	320	4	0		TgCkBr156	I(4), I(4), III(4), III(4), III(4)	I, I, III, I, I, III
4A3	Canguçu farm A	80	4	0		TgCkBr157	II or III(4), III(4), III(4), III(4), III(4)	I, III, III, I, III, III
4A4	Canguçu farm A	80	4	0		TgCkBr158	II or III(4), III(4), III(4), III(4), III(4)	III, III, III, III, III, III
4A5	Canguçu farm A	80	3[d]	0		TgCkBr159	u-1(3), III(3), III(3), III(3), III(3)	u-1, I, I, III, III, I
5A1	Rio Grande farm A	≥1280	4	1	23	TgCkBr160	u-1(4), I(4), III(4), III(4), III(4)	u-1, I, I, III, II, I
5A2	Rio Grande farm A	40	2[e]	0		TgCkBr161	II or III(2), III(2), III(2), III(2), III(2)	III, III, III, III, III, III
5B1	Rio Grande farm B	640	3	2	18, 22	TgCkBr162	u-1(3), I(3), III(3), III(3), III(3)	u-1, I, I, III, II, I
5B3	Rio Grande farm B	640	4	2	19, 19	TgCkBr163	u-1(4), I(4), III(4), III(4), III(4)	u-1, I, I, III, II, I
	Pooled chicken tissues[f]					TgCkBr164	II or III(1), III(1), III(1), III(1), III(1)	III, III, III, III, III, III

[a] Chickens from Turuçu were seronegative.
[b] Four mice were inoculated with tissues of each chicken.
[c] Two mice inoculated with tissues of each of these three chickens died within 6 days of inoculation and were considered to have died of other causes.
[d] One mouse died 6 days p.i. and was considered to have died of other causes.
[e] Of the four mice inoculated one died 5 days after inoculation and was discarded; of the three mice that survived one was found to be not infected with *T. gondii*.
[f] Tissues from chickens with titers of 1:10 or less were fed to cat no. 276, which shed oocysts.

genotyped with six additional genetic markers including c22-8, c29-2, L358, PK1, a new SAG2 and Apico (Su et al., 2006) to further identify isolates with high resolution. For these six markers, the targeted DNA sequences were first amplified by multiplex PCR using external primers for all markers. The external primers are: c22-8Fext, TGATGCATCCATGCGTTTAT; c22-8Rext, CCTCCACTTCTTCGGTCTCA; c29-2Fext, ACCCACTGAGCGAAAAGAAA; c29-2Rext, AGGGTCTCTTGCGCATACAT; L358-Fext, TCTCTCGACTTCGCCTCTTC; L358-Rext, GCAATTTCCTCGAAGACAGG; PK1-Fext, GAAAGCTGTCCACCCTGAAA; PK1-Rext, AGAAAGCTCCGTGCAGTGAT; SAG2-Fext, GGAACGCGAACAATGAGTTT; SAG2-Rext, GCACTGTTGTCCAGGGTTTT; Apico-Fext, TGGTTTTAACCCTAGATTGTGG and Apico-Rext, AAACGGAATTAATGAGATTTGAA. The multiplex PCR reaction was carried out in 25 μl of volume containing 1× PCR buffer, 2 mM $MgCl_2$, 200 μM each of the dNTPs, 0.10 μM each of the forward and reverse primers, 0.5 units of FastStart DNA polymerase (Roche, Indianapolis, IN) and 1.5 μl of DNA extract. The reaction mixture was treated at 95 °C for 4 min, followed by 25 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1.5 min. Multiplex PCR amplified products were 1:1 diluted in water and then used for second round of amplification (nested PCR) with internal primers for each marker separately. The internal primers are: c22-8F, TCTCTCTACGTGGACGCC and c22-8R, AGGTGCTTGGATATTCGC for marker c22-8; c29-2F, AGTTCTGCAGAGTGTCGC and c29-2R, TGTCTAGGAAAGAGGCGC for marker c29-2; L358-F2, AGGAGGCGTAGCGCAAGT and L358-R2, CCCTCTGGCTGCAGTGCT for marker L358; PK1-F, CGCAAAGGGAGACAATCAGT and PK1-R, TCATCGCTGAATCTCATTGC for marker PK1; SAG2-Fa, ACCCATCTGCGAAGAAAACG and SAG2-Ra, ATTTCGACCAGCGGGAGCAC for the new marker SAG2, and Apico-F, TGCAAATTCTTGAATTCTCAGTT and Apico-R, GGGATTCGAACCCTTGATA for marker Apico. All these internal primers were described in detail in a recent study (Su et al., 2006). The nested PCR was carried out in 25 μl of volume containing 1X PCR buffer, 2 mM $MgCl_2$, 200 μM each of the dNTPs, 0.30 μM each of the forward and reverse primers, 0.5 units of FastStart DNA polymerase and 1.5 μl of diluted multiplex PCR products. The reaction mixture was treated at 95 °C for 4 min, followed by 35 cycles of 94 °C for 30 s, 60 °C for 1 min and 72 °C for 2 min. The nested PCR products were analyzed to reveal their genotypes by the method described previously (Su et al., 2006).

3. Results

3.1. Pará state chickens

Antibodies to *T. gondii* were found in 20 chickens with titers of 1:10 in one, 1:20 in one, 1:40 in two, 1:80 in two, 1:160 in two, 1:320 in three, 1:640 in five and 1:1280 or higher in four. *T. gondii* was isolated from 15 chickens with titers of 1:20 or higher. It is remarkable that 51 of 60 (84%) of mice inoculated with tissues of 15 infected chickens acquired *T. gondii* infection indicating high density of infection in chicken tissues. Thirty-one of 51 (60.7%) mice that became infected after inoculation with tissues of infected chickens died of toxoplasmosis, mostly due to toxoplasmic pneumonia. All infected mice from four strains died of toxoplasmosis (Table 1). The *T. gondii* isolates were designated as TgCkBr 107–116 and 141–145 (Table 1). The cat fed seronegative chickens did not shed oocysts.

Genotyping of these 15 isolates using polymorphisms at the loci SAG1, SAG2, SAG3, BTUB and GRA6 did not find mixed infection in chickens. The results of genotyping by all 11 markers are summarized in Tables 1 and 3. Eleven genotypes were revealed for the 15 isolates. All genotypes contained different combinations of allele I, II and III. Some genotypes had unique alleles (u-1) at loci SAG1 and c22-8. No clonal types I, II and III genotypes were found.

3.2. Rio Grande do Sul

Antibodies to *T. gondii* were found in 19 (38.0%) of 50 chickens with titers of 1:20 in one, 1:40 in two, 1:80 in five, 1:160 in three, 1:320 in three, 1:640 in three and 1:1280 or higher in two.

T. gondii was isolated in mice inoculated with tissues from 18 of 19 chickens with titers of 1:20 or higher. These isolates were designated as TgCkBr146-163 (Table 2). Of the 72 mice inoculated with tissues of infected chickens seven died within 6 days p.i and were considered to have died of bacterial infections. Of the remaining 65 mice 64 were found to be infected with *T. gondii* (Table 2). All 20 infected mice from five isolates died of acute toxoplasmosis between 12 and 16 days p.i. The cat (no. 276) fed tissues from 31 seronegative chickens shed *T. gondii* oocysts. The two mice fed oocysts from cat 276 died of acute toxoplasmosis 4 days later and numerous tachyzoites were found in their mesenteric lymph nodes; these tachyzoites were infective to mice by the subcutaneous route; this isolate was designated as TgCkBr164.

Table 3
Summary of genotyping

Genotype												
Pará	SAG1	SAG2	SAG3	BTUB	GRA6	c22-8	c29-2	L358	PK1	SAG2 (new)	Apico	Isolates
1	I	III	I	III	III	III	III	III	I	III	I	TgCkBr110
2	I	III	I	III	III	III	III	III	I	III	III	TgCkBr113
3	u-1	II	III	III	II	II	nd	II	II	II	I	TgCkBr116
4	I	I	I	I	I	u-1	I	I	III	I	III	TgCkBr141
5	I	I	III	III	II	u-1	III	III	III	II	I	TgCkBr143
6	I	I	III	I	II	u-1	I	I	I	I	I	TgCkBr144
7	I	I	I	I	I	II	I	III	I	I	III	TgCkBr115, 142, 145
8	I	I	III	I	III	II	I	III	III	II	I	TgCkBr114
9	I	III	III	III	II	u-1	I	I	I	III	III	TgCkBr107, 108
10	I	I	I	III	III	II	III	I	III	II	III	TgCkBr109
11	I	III	III	III	III	III	III	III	III	III	I	TgCkBr111, 112
Rio Grande do Sul												
1	I	I	I	I	I	I	I	I	I	I	I	TgCkBr146
2	II or III	III	III	III	III	III	III	III	III	III	III	TgCkBr158, 161, 164
3	u-1	I	III	III	III	u-1	I	I	III	II	I	TgCkBr147, 148, 151, 154, 160, 162, 163
4	II or III	III	III	III	III	I	III	III	I	III	III	TgCkBr149, 150, 152, 157
5	I	III	III	III	III	III	I	III	III	III	III	TgCkBr153
6	u-1	III	III	III	III	u-1	I	I	III	III	I	TgCkBr155, 159
7	I	I	III	III	III	I	I	III	I	I	III	TgCkBr156

nd, data not available.

Genotyping of these 19 isolates using polymorphisms at the loci SAG1, SAG2, SAG3, BTUB and GRA6 did not find mixed infection in chickens. The result of genotyping by all 11 markers is presented in Tables 2 and 3. Seven genotypes were identified among the 19 isolates. Five genotypes contain different combinations of allele I, II and III. Isolate TgCkBr146 had type I allele at all 11 loci, while isolates TgCkBr158, 161 and 164 had type III allele for all markers, suggesting these isolates may be identical or very closely related to clonal types I or III lineages.

4. Discussion

Two important conclusions can be drawn from our study. First, the genetic makeup of *T. gondii* in Brazil is highly diverse. From a total of 34 chicken isolates, 18 different genotypes were identified by 11 genetic markers. There were 11 different genotypes among the 15 isolates from Pará, and seven genotypes among 19 isolates from Rio Grande do Sul. Such a high genetic diversity within a relatively small number of isolates is unusual. Our result supports findings from a few recent studies in that *T. gondii* in Brazil is diverse and genetically different from that of North America and Europe (Ferreira et al., 2006; Khan et al., 2006; Lehmann et al., 2004, 2006). At present, it is not clear the level of diversity in Brazil is the result of frequent genetic recombination among a limited pool of alleles or from a large number of lineages in the background. Further study by multilocus DNA sequencing for nuclear and organelle genomes will facilitate our understanding of *T. gondii* population in this region.

Second, *T. gondii* populations are distinct among different locations in Brazil. In this study, there are no overlapping genotypes between the states of Pará and Rio Grande do Sul, suggesting the two populations were separated and there was no genetic flow between them. This is in sharp contrast to the parasite population in North America and Europe, where one genotype (type II) predominate broad geographical regions (Ajzenberg et al., 2002; Dardé et al., 1992; Howe and Sibley, 1995). To fully understand *T. gondii* population structure in Brazil, it is necessary to collect a large number of samples that cover the whole region with high density. It is interesting to point out that we do see clonal types I- and III-like genotypes in this study. Isolate TgCkBr146 from Rio Grande do Sul had type I allele at all 11 genetic loci, and isolates TgCkBr158, 161 and 164 had type III alleles for all loci. This suggests that types I and III lineages or their close relatives are circulating in Rio Grande do Sul. After all, our data indicates that South America, the largest tropical forest region, provides an

unique niche for studying *T. gondii* population genetics and molecular evolution.

Acknowledgements

S.M. Gennari is in receipt of a fellowship from CNPq, Brazil. We thank Katherine Hopkins for technical assistance.

References

Ajzenberg, D., Banuls, A.L., Tibayrenc, M., Darde, M.L., 2002. Microsatellite analysis of *Toxoplasma gondii* shows considerable polymorphism structured into two main clonal groups. Int. J. Parasitol. 32, 27–38.

Bahia-Oliveira, L.M.G., Jones, J.L., Azevedo-Silva, J., Alves, C.C.F., Oréfice, F., Addiss, D.G., 2003. Highly endemic, waterborne toxoplasmosis in north Rio de Janeiro State, Brazil. Emerg. Infect. Dis. 9, 55–62.

Dardé, M.L., Bouteille, B., Pestre-Alexandre, M., 1992. Isoenzyme analysis of 35 *Toxoplasma gondii* isolates and the biological and epidemiological implications. J. Parasitol. 78, 786–794.

de Moura, L., Bahia-Oliveira, L.M.G., Wada, M.Y., Jones, J.L., Tuboi, S.H., Carmo, E.H., Ramalho, W.M., Camargo, N.J., Trevisan, R., Graça, R.M.T., da Silva, A.J., Moura, I., Dubey, J.P., Garrett, D.O., 2006. Waterborne outbreak of toxoplasmosis, Brazil, from field to gene. Emerg. Infect. Dis. 12, 326–329.

Dubey, J.P., 1998. Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. Vet. Parasitol. 74, 75–77.

Dubey, J.P., 2004. Toxoplasmosis-a waterborne zoonosis. Vet. Parasitol. 126, 57–72.

Dubey, J.P., Beattie, C.P., 1988. Toxoplasmosis of Animals and Man. CRC Press, Boca Raton, Florida, pp. 1–220.

Dubey, J.P., Desmonts, G., 1987. Serological responses of equids fed *Toxoplasma gondii* oocysts. Equine Vet. J. 19, 337–339.

Dubey, J.P., Graham, D.H., Blackston, C.R., Lehmann, T., Gennari, S.M., Ragozo, A.M.A., Nishi, S.M., Shen, S.K., Kwok, O.C.H., Hill, D.E., Thulliez, P., 2002. Biological and genetic characterisation of *Toxoplasma gondii* isolates from chickens (*Gallus domesticus*) from São Paulo, Brazil: unexpected findings. Int. J. Parasitol. 32, 99–105.

Dubey, J.P., Graham, D.H., Silva, D.S., Lehmann, T., Bahia-Oliveira, L.M.G., 2003a. *Toxoplasma gondii* isolates of free-ranging chickens from Rio de Janeiro, Brazil: mouse mortality, genotype, and oocyst shedding by cats. J. Parasitol. 89, 851–853.

Dubey, J.P., Navarro, I.T., Graham, D.H., Dahl, E., Freire, R.L., Prudencio, L.B., Sreekumar, C., Vianna, M.C., Lehmann, T., 2003b. Characterization of *Toxoplasma gondii* isolates from free range chickens from Paraná, Brazil. Vet. Parasitol. 117, 229–234.

Dubey, J.P., Gennari, S.M., Labruna, M.B., Camargo, L.M.A., Vianna, M.C.B., Marcet, P.L., Lehmann, T., 2006a. Characterization of *Toxoplasma gondii* isolates in free-range chickens from Amazon, Brazil. J. Parasitol. 92, 36–40.

Dubey, J.P., Patitucci, A.N., Su, C., Sundar, N., Kwok, O.C.H., Shen, S.K., 2006b. Characterization of *Toxoplasma gondii* isolates in free-range chickens from Chile, South America. Vet. Parasitol. 140, 76–82.

Ferreira, A.M., Vitor, R.W.A., Gazzinelli, R.T., Melo, M.N., 2006. Genetic analysis of natural recombinant Brazilian *Toxoplasma gondii* strains by multilocus PCR-RFLP. Infect. Genet. Evol. 6, 22–31.

Glasner, P.D., Silveira, C., Kruszon-Moran, D., Martins, M.C., Burnier, M., Silveira, S., Camargo, M.E., Nussenblatt, R.B., Kaslow, R.A., Belfort, R., 1992. An unusually high prevalence of ocular toxoplasmosis in southern Brazil. Am. J. Ophthalmol. 114, 136–144.

Howe, D.K., Sibley, L.D., 1995. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. J. Infect. Dis. 172, 1561–1566.

Jones, J.L., Muccioli, C., Belfort, R., Holland, G.N., Roberts, J.M., Silveira, C., 2006. Recently acquired *Toxoplasma gondii* infection, Brazil. Emerg. Infect. Dis. 12, 582–587.

Khan, A., Jordan, C., Muccioli, C., Vallochi, A.L., Rizzo, L.V., Belfort Jr., R., Vitor, R.W.A., Silveira, C., Sibley, L.D., 2006. Genetic divergence of *Toxoplasma gondii* strains associated with ocular Toxoplasmosis, Brazil. Emerg. Infect. Dis. 12, 942–949.

Lehmann, T., Graham, D.H., Dahl, E.R., Bahia-Oliveira, L.M.G., Gennari, S.M., Dubey, J.P., 2004. Variation in the structure of *Toxoplasma gondii* and the roles of selfing, drift, and epistatic selection in maintaining linkage disequilibria. Infect. Genet. Evol. 4, 107–114.

Lehmann, T., Marcet, P.L., Graham, D.H., Dahl, E.R., Dubey, J.P., 2006. Globalization and the population structure of *Toxoplasma gondii*. Proc. Natl. Acad. Sci. 103, 11423–11428.

Sobral, C.A., Amendoeira, M.R.R., Teva, A., Patel, B.N., Klein, C.H., 2005. Seroprevalence of infection with *Toxoplasma gondii* in indigenous Brazilian populations. Am. J. Trop. Med. Hyg. 72, 37–41.

Su, C., Zhang, X., Dubey, J.P., 2006. Genotyping of *Toxoplasma gondii* by multilocus PCR-RFLP markers: a high resolution and simple method for identification of parasites. Int. J. Parasitol. 36, 841–848.